

## Effect of benomyl and carbofuran on *Aphelenchoides besseyi* on rice <sup>(1)</sup>

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**Summary** – The efficiency of benomyl applied as seed treatment and spray and that of carbofuran applied in floodwater were tested to prevent infestation of rice plants by *Aphelenchoides besseyi* when the nematode was introduced into floodwater at different growth stages of the crop. When *A. besseyi* was inoculated at transplanting, benomyl effectively controlled it when applied as seed treatment and sprayed 1 or 15 days after transplanting. Benomyl failed to protect the plants when applied as seed treatment, and/or sprayed 5 days after nematode inoculation at transplanting, maximum tillering, and/or panicle initiation. Carbofuran broadcast in floodwater 1 or 30 days after nematode inoculation at transplanting failed to control the nematode. In addition to hot water treatment of the seeds, a combination of benomyl seed treatment and spraying 1 or 15 days after transplanting may be used to protect rice plants from infestation by *A. besseyi* that survive in the field and to produce seeds free of *A. besseyi*.

**Résumé** – *Effets du benomyl et du carbofuran sur l'infestation de plants de riz par Aphelenchoides besseyi* – Le traitement des semences et des pulvérisations de benomyl ainsi que des applications de carbofuran dans l'eau d'irrigation ont été testées pour prévenir l'infestation des plants de riz lorsque le nématode *Aphelenchoides besseyi* était introduit dans l'eau d'irrigation à différents stades de développement de la culture. Lorsque le nématode était inoculé au repiquage, le traitement des semences combiné avec une pulvérisation de benomyl effectuée 1 ou 15 jours après le repiquage contrôlaient efficacement le nématode. *A. besseyi* n'était pas contrôlé lorsque le benomyl était appliqué en pulvérisation cinq jours après l'inoculation du nématode lorsque ce dernier était inoculé au repiquage, au tallage maximum et/ou au stade de l'initiation des panicules. Des applications de carbofuran dans l'eau d'irrigation 1 ou 15 jours après l'inoculation du nématode ne contrôlaient pas ce dernier. Un traitement des semences au benomyl combiné avec une pulvérisation de ce produit 1 ou 15 jours après le repiquage peut être utilisé pour limiter l'infestation par les nématodes survivant dans le champ lorsque l'on tente de produire des graines indemnes d'*A. besseyi*.

**Key-words** : *Aphelenchoides besseyi*, benomyl, carbofuran, rice, seed production.

Hot water treatment of seeds and planting of resistant cultivars have strongly reduced yield losses caused by *Aphelenchoides besseyi* Christie, 1942 (Hollis & Keoboonrueng, 1984). However, *A. besseyi* still causes damage in some areas (Rahman & Miah, 1989) and, as a seed transmitted pathogen, is subject to quarantine regulation. Seeds exchanged between countries must be free of *A. besseyi*.

The International Rice Research Institute (IRRI) is maintaining a rice germplasm collection of more than 80 000 accessions including rice cultivars, wild rices, and species of genera related to rice. Upon request, seeds are supplied to rice scientists worldwide. Hot water treatment of seeds (Atkins & Todd, 1959) can be used to destroy the nematode infesting the seeds. This treatment does not affect seed viability and vigor when seeds are sown immediately after being treated. Un-

fortunately, it cannot be given to all seed lots sent abroad because viability and vigor of the seeds are strongly reduced when they are stored 1-4 months after treatment (Malabanan, 1987). In 1992, IRRI has exported 125 893 seed lots representing 8904 accessions outside the Philippines. All accessions were tested for the presence of *A. besseyi* and 668 were found infested. Of these, 622 were treated with hot water before shipment and 46 were rejected. Some of these accessions were valuable rare varieties available in small quantities. To avoid the loss of valuable seed lots and to counter the low seed viability and vigor brought by the hot water treatment, it would be useful to develop other methods of seed production that would provide seeds free of the nematode.

Since *A. besseyi* is mainly seedborne (Fortuner & Orton Williams, 1975), treating the seeds with hot water before sowing would efficiently reduce the risk of in-

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festation coming from contaminated seeds. There are, however, secondary sources of infestation. *A. besseyi* may survive in plant debris of the preceding crop (Sivakumar, 1987) or they may be carried by irrigation water (Tamura & Kegasawa, 1958). Methods that would prevent infestation of the plants from these secondary sources of inoculum must be developed.

Soil application of carbofuran (Rao *et al.*, 1986) has been reported to reduce *A. besseyi* infestation. Benomyl has also been reported to efficiently control *A. besseyi* (Templeton *et al.*, 1971) as well as other nematodes (Miller, 1969; Miller & Taylor, 1970; McGuire & Goode, 1970; Laughlin & Vargas, 1972; McLeod, 1973; Hide & Corbet, 1974; Cavalier *et al.*, 1987).

Two experiments were conducted under screenhouse conditions to test the efficiency of carbofuran applied into the floodwater and benomyl applied as seed treatment and sprays to prevent infestation of rice plants by *A. besseyi* when the nematode is introduced in floodwater.

### Materials and methods

Experiments were conducted in a screenhouse using the breeding line IR34686-56-2-2 that has been found severely infested by *A. besseyi* on the IRRRI farm.

In a preliminary experiment, the efficiency of three hot water treatments to control seed-transmitted *A. besseyi* were tested using 32 lots of 500 seeds. Eight seed lots were not treated. The remaining 24 seed lots were soaked in water at 25 °C for 1 h and then divided into three groups of eight lots. These lots were treated with hot water for 15 min at 50, 55 or 60 °C. All lots were air-dried at 25 °C for a day. From each seed lot, 50 seeds were manually dehulled and placed on Baermann funnels for five days and the nematodes were then counted. In addition, three seeds from each seed lot were sowed in clay pots filled with 10 dm<sup>3</sup> of soil previously sterilized for 2 h at 120 °C. Plants were grown until maturity. At harvest, 50 seeds from each plant were manually dehulled and nematodes were extracted. Treatments were arranged in randomized complete block design with eight replications.

For the two experiments testing benomyl and carbofuran for control of *A. besseyi* introduced in floodwater, seeds were treated with hot water at 55 °C for 15 min and germinated on germination paper. Seven-day-old seedlings were transplanted in clay pots (three seedlings per pot) containing 10 dm<sup>3</sup> of soil previously sterilized for 2 h at 120 °C in an autoclave. Plants were grown under permanent flooded conditions for the whole duration of the experiments. Ammonium sulfate was applied at the rate of 80 kg N/ha split in two equal applications at transplanting and at panicle initiation. Thiodan was sprayed three times to control defoliators and rice bugs. Plants were harvested 120 days after transplanting. *A. besseyi* was derived from a culture maintained on *Al-*

*ternaria tenuis* grown on potato dextrose agar at room temperature (25–28 °C).

In the first experiment, 3000 nematodes were inoculated per pot into floodwater covering the soil. Carbofuran 3G was applied in the floodwater at the rate of 4 kg a.i./ha 1 or 30 days after transplanting (DAT). Benomyl (Benlate 50 WP<sup>®</sup>) was applied as seed treatment using slurry method at the rate of 0.3 % by seed weight and/or as sprayed on the plant using a hand sprayer at the recommended rate of 2.5 g/dm<sup>3</sup> of water 1 and/or 15 DAT. Eight treatments were applied: *i*) control without nematode and chemical treatment; *ii*) control with 3000 nematodes and without chemical treatment; *iii*) 3000 nematodes and carbofuran at 1 DAT; *iv*) 3000 nematodes and carbofuran at 30 DAT; *v*) 3000 nematodes and benomyl as seed treatment; *vi*) 3000 nematodes and benomyl sprayed at 1 DAT; *vii*) 3000 nematodes and benomyl as seed treatment and benomyl sprayed at 1 DAT; and *viii*) 3000 nematodes and benomyl as seed treatment and benomyl sprayed at 1 and 15 DAT. Treatments were arranged in a randomized complete block design with eight replications.

In the second experiment, 2000 nematodes were inoculated per pot into floodwater covering the soil at transplanting, at maximum tillering, and/or at panicle initiation. Benomyl treatments consisted of *i*) absence of treatment, *ii*) application as seed treatment, and *iii*) application as spray five days after maximum tillering and/or as spray five days after panicle initiation. All combinations of inoculation date and benomyl applications were performed, resulting in twenty different treatments. Treatments were arranged in a randomized complete block design with seven replications.

At harvest, the number of panicles, the weight of filled and unfilled grains, and the weight of 100 filled grains were recorded for both experiments. For each plant, 50 filled grains manually dehulled and 3 g of unfilled grains were placed in modified Baermann funnels for five days. Nematodes were then counted.

Results of the three experiments were analyzed using ANOVA and Duncan's multiple range test (DMRT).

### Results

*A. besseyi* was not recovered from seeds which received hot water treatment at 55 or 60 °C (Table 1). Similarly, *A. besseyi* was not observed in seeds produced by plants originating from seeds subjected to these treatments. *A. besseyi* was, however, observed in seeds subjected to hot water treatment at 50 °C and in seeds of plants obtained from seeds subjected to this treatment (Table 1).

No difference in grain yield, number of panicles, weights of filled and unfilled grains, and weight of 100 filled grains was observed among treatments in both experiments. However, symptoms such as production of secondary tillers and distorted and wrinkled leaf margins were observed on most infected plants.

**Table 1.** Number of *Aphelenchoides besseyi* recovered from 50 seeds given different hot water treatments and from 100 grains harvested from plants grown from untreated and hot water-treated seeds.

Treatment	<i>A. besseyi</i> (No/50 seeds) *	<i>A. besseyi</i> (No/100 grains)
None (control)	354 a	25.6 a
50 °C for 15 minutes	37 b	6.1 b
55 °C for 15 minutes	0 b	0 b
60 °C for 15 minutes	0 b	0 b

Means of height replication. In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT.

*A. besseyi* was not detected in grains harvested from control plants (Table 2). An average of 277 and 740 *A. besseyi* were extracted from 3 g of unfilled grains and 50 filled grains, respectively. After a carbofuran treatment applied at 1 or 30 DAT, the average number of *A. besseyi* extracted from unfilled and filled grains was not significantly different from that in the control. With benomyl seed treatment alone, the average number of *A. besseyi* extracted from filled grains was significantly lower ( $p < 0.05$ ) than those without chemical treatment; in unfilled grains, the difference was not significant. The average number of *A. besseyi* recovered from unfilled and filled grains harvested from plants sprayed once with benomyl was significantly lower ( $p < 0.05$ ) than those observed in the absence of chemical treatment and in carbofuran-treated plants. *A. besseyi* was not detected in grains harvested from plants given benomyl seed treatment and sprayed at 1 and 15 DAT.

The average number of *A. besseyi* recovered from filled grains harvested from plants inoculated at transplanting and whose seeds were treated with benomyl was significantly lower ( $p < 0.05$ ) than those observed in any other benomyl treatments (Table 3). No significant difference in the number of *A. besseyi* obtained from filled or unfilled grains was observed among treatments.

The average number of *A. besseyi* extracted from filled grains was constantly significantly lower ( $p < 0.05$ ) when plants were inoculated at panicle initiation rather than at transplanting and maximum tillering. An exception was when benomyl was applied as seed treatment at transplanting (Table 3).

## Discussion

Hot water treatment of the seeds at 55 or 60 °C for 15 min was effective in preventing seed transmission of *A. besseyi*. In both experiments, *A. besseyi* inoculated in the floodwater at transplanting, maximum tillering, panicle initiation, or at all three stages were able to parasitize

the plant and infest panicles and grains. These observations indicate that under field conditions, individuals of *A. besseyi*, surviving in the field on weeds, rice husks, or plant debris of the previous crop (Yoshii & Yamamoto, 1950; Tikhonova, 1966; Sivakumar, 1987) or carried by irrigation water (Tamura & Kegasawa, 1958) from neighboring infested fields can also infest plants obtained from seeds treated with hot water.

**Table 2.** Effect of carbofuran and benomyl treatments applied at different times after transplanting and of inoculation of *Aphelenchoides besseyi* on average number of nematodes observed in unfilled spikelets and filled grains at harvest (experiment 1).

Treatment	Average number of <i>Aphelenchoides besseyi</i> observed in	
	3 g of unfilled spikelets	50 filled grains
Control	0 a	0 a
3000 <i>A. besseyi</i>	277 bc	740 c
3000 <i>A. besseyi</i> , carbofuran at 1 DAT *	230 bc	625 bc
3000 <i>A. besseyi</i> , carbofuran at 30 DAT	378 c	577 bc
3000 <i>A. besseyi</i> , benomyl seed treatment	116 ab	325 ab
3000 <i>A. besseyi</i> , benomyl sprayed at 1 DAT	14 a	0 a
3000 <i>A. besseyi</i> , benomyl seed treatment and sprayed at 1 DAT	3 a	0 a
3000 <i>A. besseyi</i> , benomyl seed treatment and sprayed at 1 and 15 DAT	0 a	0 a

Means of eight replications. In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT.

\* DAT = days after transplanting.

**Table 3.** Effect of different time and mode of application of benomyl and different inoculation time on average number of *Aphelenchoides besseyi* detected in 50 filled grains at harvest (experiment 2).

Benomyl treatment	Inoculation time			
	Transplanting	Maximum tillering	Panicle initiation	All three stages
No treatment, control	85 ab, A	82 a, A	14 a, B	109 a, A
Seed treatment	52 b, B	119 a, A	24 a, B	112 a, A
Spraying at 5 DAMT *	96 a, A	88 a, A	29 a, B	79 a, A
Spraying at 5 DAPI **	107 a, A	113 a, A	11 a, B	129 a, A
Seed treatment and spraying at 5 DAMT and 5 DAPI	42 b, BC	80 a, AB	14 a, C	117 a, A

\* DAMT = days after maximum tillering, \*\* DAPI = days after panicle initiation.

In a column average number followed by a common lower case letter are not significantly different at the 5 % level by DMRT. In a row average number followed by a common upper case letter are not significantly different at the 5 % level by DMRT.

In these experiments, *A. besseyi* did not affect grain yield and yield components of IR34686-56-2-2-2. This line appears to be tolerant to *A. besseyi* even if infected plants showed symptoms of infestation.

Carbofuran applied in floodwater failed to control *A. besseyi*. Even when carbofuran was applied one day after inoculation, it did not reduce the number of *A. besseyi* observed in grains at harvest.

Benomyl applied as seed treatment reduced the average number of *A. besseyi* present in filled grains at harvest. However, it did not totally prevent infestation. Benomyl applied as spray one day after inoculation of the nematodes appeared to control *A. besseyi* more effectively than when applied as seed treatment. Total control of *A. besseyi* was obtained when benomyl was applied as seed treatment, followed by two sprayings at one and fifteen days after nematode inoculation. When the first spraying of benomyl was delayed by five days after *A. besseyi* infestation, the nematode was not controlled. The relative inefficiency of benomyl as seed treatment to control the nematode may indicate that it does not have a systemic effect against *A. besseyi* as has been reported for sedentary endoparasitic nematodes (Cavelier *et al.*, 1987). It seems that to control *A. besseyi* efficiently, benomyl must be applied during the infestation stage. Benomyl becomes inefficient once the nematode starts feeding on the apical meristematic tissues within the innermost leaf sheath. This also indicates that benomyl could inhibit the migration of *A. besseyi* toward the growing point of the stem in much the same way that benomyl inhibits the penetration of *Globodera rostochiensis* (Cavelier *et al.*, 1987).

When *A. besseyi* inoculum is present in the fields at transplanting time, it may be possible to reduce the infestation of the young seedling by applying benomyl as seed treatment and spraying immediately after transplanting. This is in addition to the hot water seed treatment that controls *A. besseyi* in the seeds. These treatments may be useful in producing seeds of valuable cultivars, when it is necessary to export seeds which are free of the nematodes. These treatments will most certainly not be of economic significance when used for regular rice production in farmers' fields. When the inoculum of *A. besseyi* is introduced in the field through irrigation water at a later stage of the crop, it will be difficult to reduce plant infestation by using benomyl spraying because infestation time cannot be predicted.

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